Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE
1969, v131, 858-860

Antigenicity of Psittacosis Vaccines Killed by Ionizing Radiation (33994)

GEORGE G. WRIGHT, NORMAN S. SWACK,[1] AND JOHN R. MITZEL
Medical Sciences Laboratory, Fort Detrick, Frederick, Maryland 21701

Nonviable psittacosis vaccines killed by the action of formaldehyde have been derived from preparations of the agent propagated in tissues of animals, in embryonated eggs, or in tissue culture (1–3). Preparations of high initial infectivity yielded vaccines that provoked significant protection against intraperitoneal challenge in mice and other animals. Protection was less effective against respiratory challenge, although vaccines propagated in human diploid cell cultures were evidently more effective than earlier preparations (3). Further increase in antigenicity would be desirable to increase the level and duration of immunity, or to obviate the requirement for adjuvants or for multiple-dose immunization. Recent studies showed that vaccines for a variety of agents killed by ionizing radiation retain greater antigenicity than vaccines killed by chemicals or heat (4–6). Observations on the applicability of this method to preparation of psittacosis vaccines are presented below.

Materials and Methods. Tissue culture. Four- to 6-day-old monolayers of the human diploid cell strain WI-38 (7) were used for propagation of agent for preparation of vaccines. Cultures were used between passages 20 and 30.

Agent strain. The Borg or Louisiana strain of *Chlamydia psittaci* was obtained from the Virus and Rickettsia Division, Fort Detrick, as the fourth yolk-sac passage. The sixth yolk-sac passage was used both for seed inoculum and challenge of mice.

Suspensions of Borg agent. Cell monolayers were inoculated with Borg agent and after incubation for 2 hr at 37° were washed with balanced salt solution (BSS) (8). Maintenance medium was then added, consisting of basal medium (9) prepared with BSS and

[1] Present address: Virology Laboratory, Veterans Administration Hospital, West Haven, Connecticut 06516.

TABLE I. Effect of Radiation Dose on Antigenicity of Suspensions of Borg Agent in Mice.

State during irradiation	Dose of radiation 10^6 R	Infectivity of challenge stock for mice immunized with respective preparation[a]			
		Log_{10} LD_{50} /0.5 ml	Variance of LD_{50}	95% Confidence limits	Protective index[b]
Fluid	0.5	4.40	0.116	3.73–5.06	3.61
	1.0	4.86	0.301	3.52–6.20	3.15
	2.0	5.23	0.315	3.86–6.60	2.78
	4.0	5.67	0.141	4.93–6.40	2.34
Frozen	0.5	3.45	0.175	2.64–4.27	4.56
	1.0	3.98	0.110	3.33–4.63	4.03
	2.0	3.43	0.577	1.48–5.39	4.58
	4.0	3.61	0.148	2.86–4.37	4.40
Formaldehyde, 0.02%		4.65	0.0601	4.17–5.13	3.36
Unimmunized control mice		8.01	0.0648	7.52–8.51	

[a] Mice were immunized by one ip injection of 0.5 ml of suspension and challenged after 3 weeks.

[b] Protective index equals log_{10} mouse $IPLD_{50}$/ml of challenge suspension in control mice minus log_{10} mouse $IPLD_{50}$/ml of same suspension in respective immunized mice.

supplemented with 3% calf serum. After 18 hr at 37°, the medium was replaced with fresh maintenance medium. After a further 3–4 days of incubation, the cultures showed a characteristic cytopathic effect. The supernatant fluids were collected, pooled, and clarified by low-speed centrifugation. Infectivity titers were estimated by injecting 0.2-ml quantities of serial tenfold dilutions into the yolk sac of 6-day-old embryonated chicken eggs. Ten eggs were used for each level, and deaths were recorded for 10 days. End points were calculated by the method of Reed and Muench (10) or, for data in Table I, by probit analysis. Procedures for preparation of formaldehyde-treated vaccines have been reported previously (3, 11).

Irradiation. Suspensions were irradiated at the National Bureau of Standards through the courtesy of Daniel W. Brown. Suspensions of Borg agent were exposed to γ radiation from a ^{60}Co source at a dose rate of 10^5R/min. The suspensions were irradiated in 100-ml quantities in polycarbonate centrifuge bottles. Samples irradiated in the frozen state were frozen in an alcohol plus dry ice bath, packed in dry ice during irradiation, and held in the frozen state until tested for infectivity. Samples irradiated in the fluid state were stored at refrigerator temperature but were not frozen.

Antigenicity determinations. Male mice of the Ragg strain of Swiss Webster weighing 12–g were obtained from the Fort Detrick colony. The animals were immunized by ip injection of 0.5 ml of vaccine and challenged ip after 3 weeks using 10 mice/level. Immunized mice and normal controls were challenged by injection of serial tenfold dilutions of infected yolk-sac suspension into groups of animals. Animals were observed daily for 14 days after challenge. The difference between log_{10} LD_{50}/ml in control and in vaccinated mice was reported as the protective index.

Results and Discussion. The loss of infectivity of Borg agent as a function of dose of radiation was studied in fluid and in frozen suspensions. Irradiation in the frozen state reduces the secondary effects of irradiation, and it was anticipated that less destruction of antigenicity would occur under these conditions. Fluid suspensions were irradiated at room temperature, frozen suspensions were packed in dry ice. In a typical experiment, portions of a suspension were irradiated at a constant dose rate for periods such that exposures ranged from 50,000 to 400,000 R.

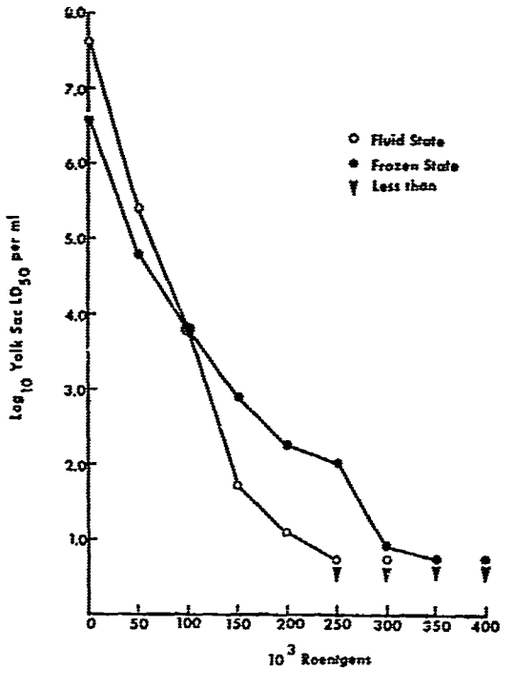

FIG. 1. Inactivation of the Borg strain of *Chlamydia psittaci* by gamma-radiation: each point represents the mean of three separate experiments. The different doses of radiation were obtained by varying the time of exposure at a constant dose rate.

The frozen samples lost approximately one $\log_{10}$ of infectivity titer as a result of freezing and thawing. Results are presented in Fig. 1. It is evident that the plots of $\log_{10}$ infectivity vs. dose of radiation deviate significantly from linearity and, accordingly, the reactions depart from first-order behavior, an effect encountered frequently in inactivation of viruses by physical and chemical agents (12). Reduction in infectivity to less than the detectable level required 250,000 R for the fluid suspension, and 400,000 R for the frozen suspension.

The effects of larger doses of radiation on antigenicity of the suspensions in mice were then investigated. Fluid and frozen suspensions were exposed to doses ranging from 500,000 to 4,000,000 R. A portion of the unirradiated suspension was treated with 0.02% formaldehyde for 4 days at 20°, followed by 10 days at 4°. The results of a typical experiment are summarized in Table I. Antigenicity of the fluid suspensions was gradually lost as the radiation was increased; frozen suspensions, however, did not lose detectable antigenicity at the highest dose of radiation tested, 4,000,000 R. This dose represents at least 10 times the level required to reduce infectivity below the detectable level. Thus, a large factor of safety is available to assure the noninfectivity of irradiated vaccine. The vaccines irradiated in the frozen state were regularly more antigenic than the formaldehyde-treated vaccines, the differences in protective index being 1.0 and 1.1 in the two experiments carried out under these conditions.

Summary.—Exposure of suspensions of the Borg strain of *Chlamydia psittaci* to graded doses of gamma radiation resulted in loss of infectivity. Exposure in the frozen state produced slower loss of infectivity than exposure in the fluid state, doses of 400,000 and 250,000 R being required, respectively, for complete loss of detectable infectivity. Both reactions deviated moderately from first-order behavior. The frozen suspensions retained greater antigenicity for mice than did the fluid suspensions and were also more antigenic than suspensions killed by treatment with formaldehyde.

The authors are indebted to Dr. James R. Gebert for statistical analysis of the data.

1. Meyer, K. F. and Eddie, B., Ann. N. Y. Acad. Sci. 98, 288 (1962).

2. Wagner, J. C., Meiklejohn, G., Kingsland, L. C., and Hiekish, H. W., J. Immunol. 54, 35 (1954).

3. Swack, N. S. and Duff, J. T., J. Infect. Diseases 118, 468 (1968).

4. Donaldson, D. M. and Mitchell, J. R., Proc. Soc. Exptl. Biol. Med. 101, 204 (1959).

5. Polley, J. R., Can. J. Microbiol. 8, 455 (1962).

6. Landay, M. E., Wright, G. G., Pulliam, J. D., and Finegold, M. J., J. Bacteriol. 96, 804 (1968).

7. Hayflick, L., Am. Rev. Respirat. Diseases 88, 387 (1963).

8. Hanks, J. H. and Wallace, R. E., Proc. Soc. Exptl. Biol. Med. 71, 196 (1949).

9. Eagle, H., Science 122, 501 (1955).

10. Reed, L. J. and Muench, H., Am. J. Hyg. 27, 493 (1938).

11. Pearson, J. W., Duff, J. T., Gearinger, N. F., and Robbins, M. L., J. Infect. Diseases 115, 49 (1965).

12. Hiatt, C. W., Bacteriol. Rev. 28, 150 (1964).

Received Feb. 28, 1969. P.S.E.B.M., 1969, Vol. 131.